



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Lecithin Lactose Agar is recommended for the isolation and differentiation of histotoxic clostridia. It is of value in the speciation of the toxin-producing members of the genus *Clostridium*.

II PERFORMANCE TEST PROCEDURE

1. Reduce all anaerobic media plates overnight at room temperature in a **BD GasPak™ EZ** anaerobic system.
2. Preparation of inocula
 - a. Prepare the anaerobe test cultures for inoculation by cultivating for 1–3 days in Chopped Meat Broth.
 - b. Use a 5-h broth culture of *S. aureus* and dilute to yield 10^3 – 10^4 CFU/plate.
3. Inoculation of the plates
 - a. Using a volumetric pipettor or equivalent method, deliver 0.05 mL of the appropriate inoculum to the plated media samples and streak for isolation.
 - b. Include **Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)** plates as controls for all organisms.
 - c. Incubate all plates anaerobically (**BD GasPak EZ System**) at $35 \pm 2^\circ\text{C}$.
4. Examine the facultative organism at 18–24 and/or 48 h. Examine the obligate anaerobes at 48 and 72 h for amount of growth, colony size, opalescence and fermentation (yellow around colonies).
5. Expected Results

Organisms	ATCC™	Recovery	Lecithinase Production	Lactose Fermentation
* <i>Clostridium histolyticum</i>	5127	Fair to heavy growth at 48 h	–	–
* <i>Clostridium perfringens</i>	13124	Fair to heavy growth at 48 h	+	+
* <i>Clostridium septicum</i>	6008	Fair to heavy growth at 48 h	–	+
* <i>Staphylococcus aureus</i>	25923	No growth		

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 6.8 ± 0.2 .
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at $35 \pm 2^\circ\text{C}$ for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Lecithin Lactose Agar is recommended for the isolation and differentiation of histotoxic clostridia from clinical specimens.¹ It is particularly useful in differentiating *Clostridium perfringens*, *C. sordellii*, *C. novyi*, *C. septicum* and *C. histolyticum*.

V SUMMARY AND EXPLANATION

Culture media containing egg yolks were useful in isolating, cultivating and identifying species of histotoxic clostridia. In 1948, McClung and Toabe reported on the use of an egg yolk medium for this purpose.¹ Willis and Hobbs added milk and lactose to the egg yolk in a formulation designed to group clostridia on the basis of production of lecithinase, hydrolysis of casein and lactose fermentation.² Neomycin sulfate was included in their formulation in order to make it a selective medium.

Willis, in response to problems in the obtaining and processing of antibiotic-free eggs, developed an egg-free medium in which purified lecithin was substituted for the egg yolk.³ In order to further refine the growth-promoting and selective properties, Ellner and O'Donnell developed the formulation, designated as Lecithin Lactose Agar in which a decreased concentration of neomycin was employed and to which sodium azide was added.⁴ This medium continues to be an important one for visualizing and characterizing the histotoxic clostridia.

VI PRINCIPLES OF THE PROCEDURE

Lecithin Lactose Agar provides differentiation of clostridia by the demonstration of lecithinase production and lactose fermentation. The medium is rendered selective by the addition of neomycin and sodium azide. Gram-negative and aerobic gram-positive rods are inhibited. Growth of gram-positive cocci is suppressed.

On this medium, the production of an opaque zone surrounding colonies indicates lecithinase production. A yellow color surrounding colonies indicates lactose fermentation due to the effect of the lowered pH on the bromocresol purple indicator.

VII REAGENTS

Lecithin Lactose Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	12.65 g	Lactose	10.0 g
Peptic Digest of Animal Tissue	5.5 g	Sodium Azide	0.2 g
Pancreatic Digest of Heart Muscle	3.3 g	Neomycin Sulfate	0.15 g
Yeast Extract	3.85 g	L-Cysteine Hydrochloride.....	0.5 g
Corn Starch	1.1 g	Calcium Chloride	0.05 g
Sodium Chloride	5.5 g	Egg Lecithin	0.66 g
Agar	15.0 g	Bromcresol Purple	25.0 mg

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁵⁻⁸ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{9,10} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Lecithin Lactose Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

As soon as possible after receipt in the laboratory, inoculate the specimen onto a reduced Lecithin Lactose Agar plate and streak for isolation. Since some anaerobes may be inhibited due to the selective nature of the medium, it is advisable to include a nonselective medium such as CDC Anaerobe Blood Agar.

Media should be reduced prior to inoculation by placing under anaerobic conditions for 6–24 h prior to use.¹¹ An efficient and easy way to obtain suitable anaerobic conditions is through the use of **GasPak** EZ anaerobic systems.

Incubate immediately under anaerobic conditions or place in a holding jar flushed with oxygen-free gas(es) until sufficient plates are accumulated (but no longer than 3 h).¹² Incubation should be at 35 ± 2°C for at least 48 h. Regardless of anaerobic system used, it is important to include an indicator of anaerobiosis such as the **GasPak** disposable anaerobic indicator.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Plates may be examined after a minimum of 48 h incubation under anaerobic conditions.

On Lecithin Lactose Agar the production of a zone of opalescence around colonies indicates lecithinase production; a yellow color around colonies indicates lactose fermentation. Reactions are listed below:

Organism	Lecithinase Production	Lactose Fermentation
<i>C. perfringens</i>	+	+
<i>C. sordellii</i>	+	–
<i>C. novyi</i>	+	–
<i>C. septicum</i>	–	+
<i>C. fallax</i>	–	+
<i>C. histolyticum</i>	–	–

Additional testing may be performed to differentiate the above species.^{13,14}

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.^{9,10,13-18}

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of

organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY

Cat. No.	Description
221858	BBL™ Lecithin Lactose Agar, Pkg. of 10 plates

XIII REFERENCES

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Becton, Dickinson and Company
7 Loveton Circle
Sparks, Maryland 21152 USA
800-638-8663

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